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Full Length Article

Differential Changes in Growth and Enzyme Activities of Chilling Induced Wheat Seedlings by Nitric Oxide Priming

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Abstract

Wheat reportedly has a positive response to seed priming in any environmental condition. The present study evaluates the changes in germination, seedling growth and antioxidant enzyme activities by nitric oxide priming (NO) in seedlings of seven wheat genotypes under normal and chilling stress. Priming was achieved by exposing the seeds of NARC-2011, AAS-2011, Punjab-2011, Faisalabad-2008, Uqab-2002, Chakwal-50 and Lasani to 10^{-4} and 10^{-5} *M* aerated sodium nitroprusside (SNP, as nitric oxide donor) solutions for 7 h. Genotypes behaved differentially in terms of growth and antioxidant response to stress and priming. Seedling growth was increased in primed seeds compared to dry seeds under control and stress conditions. The activities of superoxide dismutase, indole acetic acid oxidase, ascorbate peroxidase and guaiacol peroxidase were affected differently in almost all genotypes under stress conditions. Maximum seedling growth was revealed by SNP primed NARC-2011 and Uqab-2002 in both growth conditions. Faisalabad-2008 revealed higher antioxidant enzyme activities by SNP priming. It was concluded that response of genotypes vary under different growth conditions and different levels of SNP priming. Oxidative species scavenging system was more actively involved in detoxification of oxidative stress induced by chilling and subsequently enhanced the growth of wheat seedlings.

Keywords: Chilling stress; Wheat; Sodium nitroprusside; priming; Growth; Enzymes

Introduction

Cold stress or low temperature stress is major environmental factor affecting plant growth and yield and resulting considerable loss to crops (Farooq *et al.* 2009; Sanghera *et al.* 2011; Ahmad *et al.* 2014). Low temperature has a lethal effect on germination that results in reduced photosynthetic rate along with reduced mass of above ground organs. Cold stress (low temperature) causes numerous alterations in biochemical and physiological processes of plants (Zhao *et al.* 2009; Farooq *et al.* 2017). Generally, photosynthesis and many other physiological processes are sensitive to chilling stress, which result in the decline of growth and yield in plants. By understanding the responses of plants towards stress, crops can be made stress tolerant (Zhao *et al.* 2009). Among crops, wheat (*Triticum aestivum* L.) is an important food crop for more than one third of the world population and its yield is being influenced because of global climate change and low temperature stress in the environment (Farooq *et al.* 2008; Chaves and Oliveira 2004). Wheat often experiences cold stressed conditions during its life cycle so it is necessary to understand the natural genetic variation in characters related to stress tolerance (Loggini *et al.* 1999).

Oxidative stress is induced in the cell because of higher electron leakage towards O_2 during respiratory and photosynthetic processes leading to augmentation in generation of reactive oxygen species (ROS) which can damage to membrane proteins, DNA and lipids leading to cell death (Mittler 2002; Simova-Stoilova *et al.* 2008). During optimal conditions, balance is tightly controlled between ROS formation and its consumption by antioxidant defense system of the plant. Catalase (CAT), Superoxide dismutase (SOD) and peroxidase (POD) are key antioxidants that play a crucial role in plant defense against ROS (Noctor and Foyer 1998; Simova-Stoilova *et al.* 2008).

Many studies have revealed that increasing antioxidant defense resulted in stress tolerance to temperature extremes (Almeselmani *et al.* 2006). Zhao *et al.* (2009) reported that chilling tolerance in tomato varieties could be designated by higher activities of SOD, CAT, POX and APX enzymes.

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Studies suggest that nitric oxide (NO) has the potential to induce tolerance in plants against different environmental stresses. These investigations suggest that NO has antioxidant properties and may act as a signal to activate ROS scavenging enzymes under abiotic stress. It plays an imperative role in resistance to heavy metal, UV-B, drought, salt as well as to low and high temperature stress (Nabi *et al.* 2019). It has been accepted that NO plays a crucial role in the varied physiological functions of plants (Libourel *et al.* 2006; Zheng *et al.* 2009). Differential antioxidant defense reactions of susceptible versus resistant wheat genotypes to stress-induced oxidative stress at a specific growth stage and in controlled conditions have been reported (Loggini *et al.* 1999; Lascano *et al.* 2001).

The effect of stress on plant species depends on variety, duration and intensity of the stress in addition to developmental stage (Simova-Stoilova *et al.* 2008). There is probability that genotypes could respond variously under varied growth conditions and priming at similar growth stage. So, true chilling acclimation potential of wheat genotypes could differ under changed growth conditions and priming (Afzal *et al.* 2008). Any genotype can be more competent and specific due to better adoptive changes in metabolic and anti-oxidative processes due to hormonal (Sgherri *et al.* 2000) biochemical grounds. Therefore, present study was carried out to provide insights in the relationship of SNP priming and chilling stress tolerance of seven wheat genotypes by affecting changes in antioxidant enzymes and subsequent growth of the seedlings.

Materials and Methods

Wheat seeds of seven genotypes, *viz*. NARC-2011, AAS-2011, Punjab-2011, Faisalabad-2008, Uqab-2002, Chakwal-50 and Lasani were taken from the Agriculture Department Muzaffarabad, Azad Jammu & Kashmir, Pakistan.

Seeds were soaked in aerated solution of 10^{-4} and 10^{-5} molar sodium nitroprusside (SNP) in 100 mL glass beakers for 6 h at 25°C. After SNP priming, seeds were washed with distilled water (Bradford 1986) then dried back on dry filter papers. Three replicates of seeds (90) of each variety for each treatment were placed in Petri dishes on blotting paper at 25°C as a control and 04°C as a stress for 15 days. About 5 mL of distilled water was used to moisturize each Petri dish. Germination was counted on daily basis. After termination of germination, seedlings were measured for following parameters and then harvested for biochemical analysis.

Germination speed was measured by using equation of Rajabi and Poustini (2005). Germination percentage was determined by using the procedure and formula of AOSA (1983). Length of the shoot was taken from five randomly selected seedlings. Number of leaves was determined by counting the number of leaves from five randomly selected seedlings in each pot. Number of roots was determined by counting the number of leaves from all the studied samples by randomly selected five seedlings of each pot.

Antioxidant enzymes activity

Supernatant was used to estimate the activity of SOD by recording the decrease in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme. About 3 mL of reaction mixture was taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 μM) and placing the tubes below a light source of two 15 W florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tubes with black cloth. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of the samples to 50 percent in comparison with tubes lacking enzymes.

The activity of indole acetic acid oxidase was determined according to the method of Omran (1980); Talwar (*et al.* 1985) with some modifications. The enzyme extract was incubated for 60 min with 0.2 *M* sodium phosphate citrate buffer having pH 5.6, 200 μg mL^{-1} of IAA solution in 0.5 m*M* $MnCl_2$ and 0.1 m*M* of 2,4 dichlorophenol. Salkowski's reagent was used to stop the incubation process in addition of its reaction with unoxidized IAA. The absorbance of the sample was measured spectrophotometrically at 540 nm.

The activity of Ascorbate peroxidase was determined according to Bartoli *et al.* (1999) from the extract prepared for PPO activity. The activity of enzyme was analyzed by following the decrease in the absorbance (265 nm) of the reaction mixture having 50 μM ascorbate, 50 μM of potassium phosphate buffer, pH 6.5 and 90 μM of hydrogen peroxide.

Guaiacol peroxidase activity was determined following the method of Plewa *et al.* (1991) from the extract. Reaction mixture contained 2.77 mL of 50 m*M* Phosphate buffer (pH 7), 25 μL of enzyme extract, 0.1 mL of 1% H_2O_2 and 0.1 mL of 4% guaiacol. Increase in absorbance due to guaiacol oxidation was recorded at 470 nm.

Statistical analysis of data

Significance of the data was tested by analysis of variance and Duncan's Multiple Range Test at $P \leq 0.05$ and where applicable at $P \leq 0.01$ and $P \leq 0.03$ using MSTAT software. *Standard error* was performed to determine random error in the data

Results

Interaction of wheat varieties with SNP concentrations showed that same variety after various seed priming responded differently as varied germination speed was obtained after different SNP treatments (Fig. 1). Maximum germination speed was recorded in AAS-2011, NARC-2011

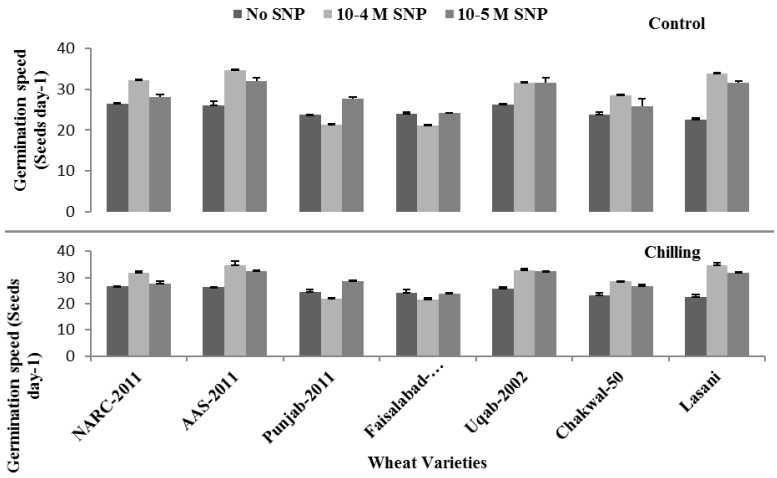

Fig. 1: Germination speed of wheat varieties as affected by SNP priming under normal and chilling conditions

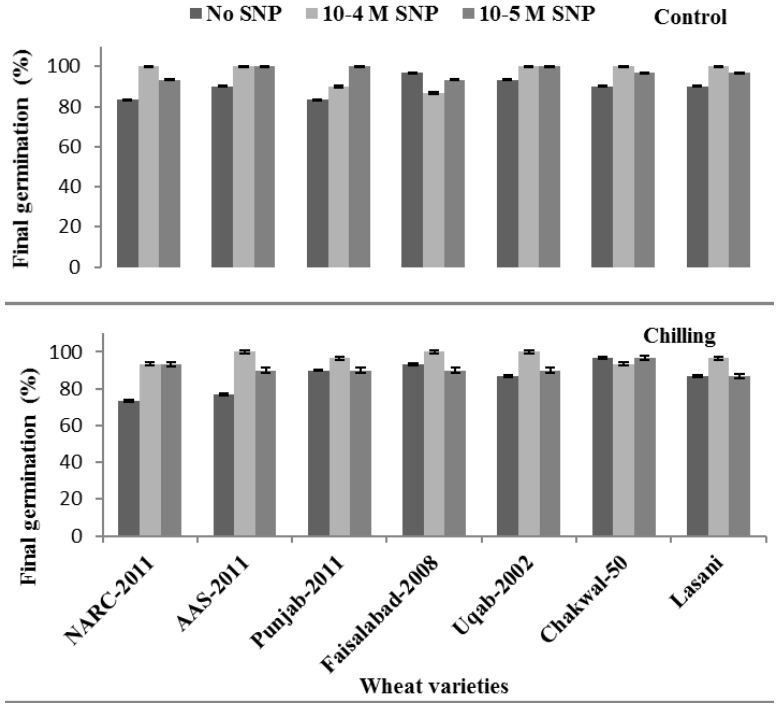

Fig. 2: Final germination of wheat varieties as affected by SNP priming under normal and chilling conditions

and Lasani seeds primed with 10^{-4} *M* SNP in normal growth condition. Minimum germination speed was recorded from 10^{-4} *M* SNP primed Punjab-2011 and Faisalabad-2008 under chilling stress. All SNP priming treatments improved final germination in all varieties except Faisalabad-2008 in controlled conditions while 10^{-4} *M* SNP priming showed maximum final germination in most of cultivars under chilling stress (Fig. 2).

Comparison of wheat varieties with varied SNP priming and growth conditions showed very highly significant variations in shoot length (Fig. 3). Maximum shoot length was recorded from 10^{-5} *M* SNP primed Uqab-2002 and NARC-2011 in normal conditions and the minimum from Chakwal-50 with 0 *M* SNP treatment in chilled conditions. Leaf numbers were determined from all the studied samples and non-significant variation was observed by wheat varieties, SNP priming and growth conditions (Fig. 4). Wheat varieties under different growth conditions and SNP concentrations revealed very highly significant variations for root numbers (Fig. 5). Unprimed Lasani gave more number of roots as compared to SNP priming treatments under chilling stress. However, SNP priming improved root number in AAS-2011 during low temperature conditions.

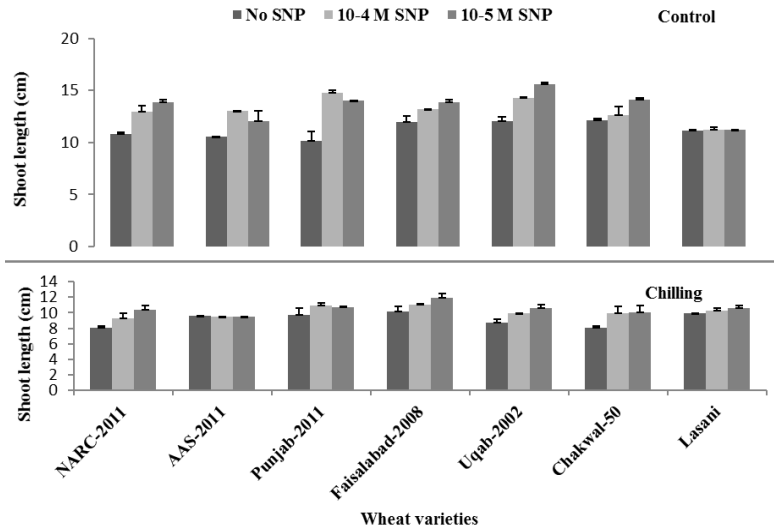

Fig. 3: Variations in shoot length of wheat varieties as affected by SNP priming and chilling

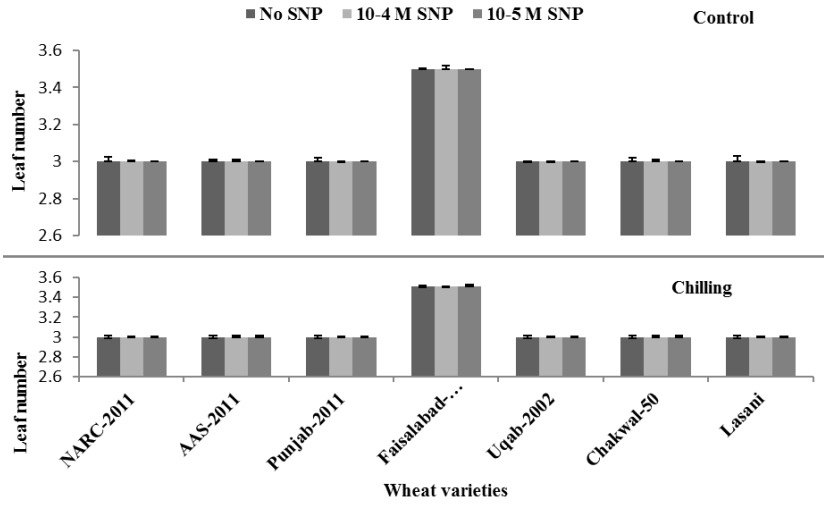

Fig. 4: Variations in leaf number of wheat varieties as affected by SNP priming and chilling

The activity of superoxide dismutase (SOD) significantly varied among wheat varieties under different growth conditions and SNP priming (Fig. 6). SNP priming significantly improved SOD activity in Punjab-2011 under controlled conditions whereas maximum SOD activity was recorded in seedlings raised from unprimed seeds of NARC and Lasani under chilling stress. Regarding indole acetic acid, maximum activity was recorded by Faisalabad-2008 with 10^{-5} *M* SNP priming in chilled growth condition (Fig. 7). Ascorbate peroxidase activity was not significantly improved in all varieties under both normal and chilling stress conditions (Fig. 8). The response of SNP priming and wheat varieties for Guaiacol peroxidase was found quite variable, however, activity was more pronounced in unprimed seeds of NARC, AAS-2011 and Uqab-2002 (Fig. 9).

Discussion

Present study revealed positive effect of NO priming and negative effect of chilling on germination traits of wheat varieties (Fig. 1–2). Negative effect of chilling is due to fact that chilling caused reduction in germination by detaining metabolic reactions and reducing water potential in germinating seeds (Bibi *et al.* 2017). Chilling stress restrains different metabolic reactions by preventing the expression of total genetic potential of the plants (Chinnusamy *et al.* 2007). Data from literature provide evidence in favor of our findings that nitric oxide regulates the response of plants towards stress (Bibi *et al.* 2017). Ansari *et al.* (2012) and Sharafizad *et al.* (2012) reported negative effect of stress and positive effect of priming on germination traits because priming has the ability to induce some metabolic changes in seed that

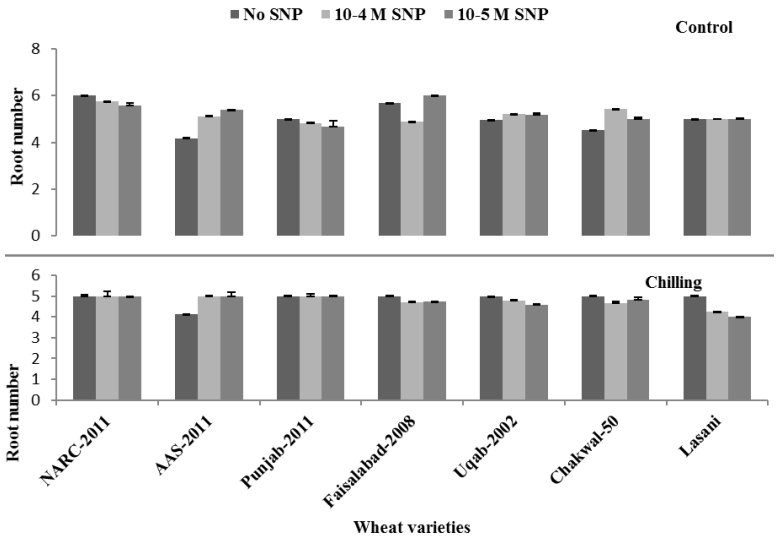

Fig. 5: Variations in root number of wheat varieties as affected by SNP priming and chilling

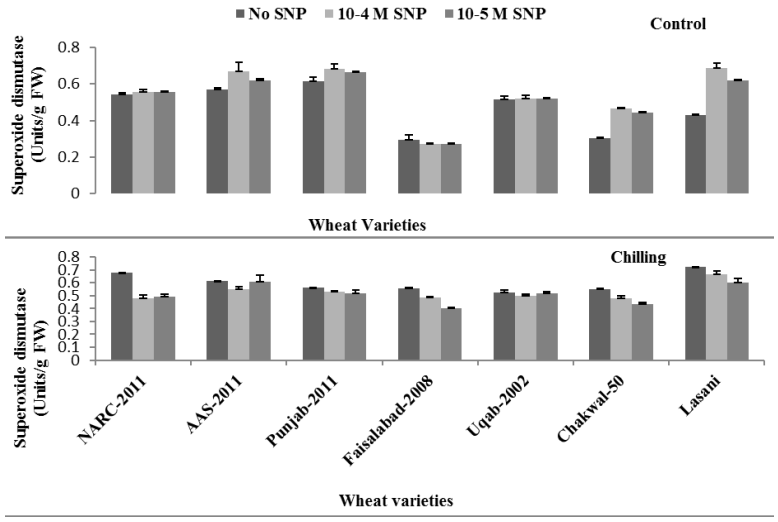

Fig. 6: Variations in superoxide dismutase of wheat varieties as affected by SNP priming and chilling

ultimately enhance its germination speed and percentage as well. During priming, uniform and rapid stimulation of some physiological changes occur in seeds that boost their speed of germination (Bradford 1986). Nitric Oxide has been implicated in promotion of seed germination in many species either by reducing seed dormancy, or by reducing the effects of adverse environmental conditions (Krasuska *et al.* 2017). SNP has been found to promote seed germination by inducing the activity of β-amylase during early stages of seed germination in many plant species such as Mouse-air cress, wheat and alfalfa (Duan *et al.* 2007; Maurice *et al.* 2016).

Different varieties have different genetic potential of growth and response; hence show variations in their morphology (Aghamolki *et al.* 2014). Chilling stress prevents expression of total genetic potential in plants leading to negative effects on plants' growth and morphology due to blockage of different metabolic reactions. The increase in plant growth i.e. shoot length, root length is linked with the SNP-mediated mitigation of chilling-induced over-production of reactive oxygen species possibly by up-regulating the antioxidative defense mechanisms. Exogenous SNP as seed priming is effective in maintaining the plant water relations by up-regulating the synthesis of Proline (Chinnusamy *et al.* 2007). Our results are in accordance with other researchers (Aghamolki *et al.* 2014; Lianopoulou and Bosabalidis 2014) who reported negative change in morphology and differences among varieties due to temperature stress. Bibi *et al.* (2018) reported positive effect of NO priming on morphological attributes in wheat varieties and adverse effects of chilling stress. Although they found non-significant variations in total number of leaves despite varied conditions.

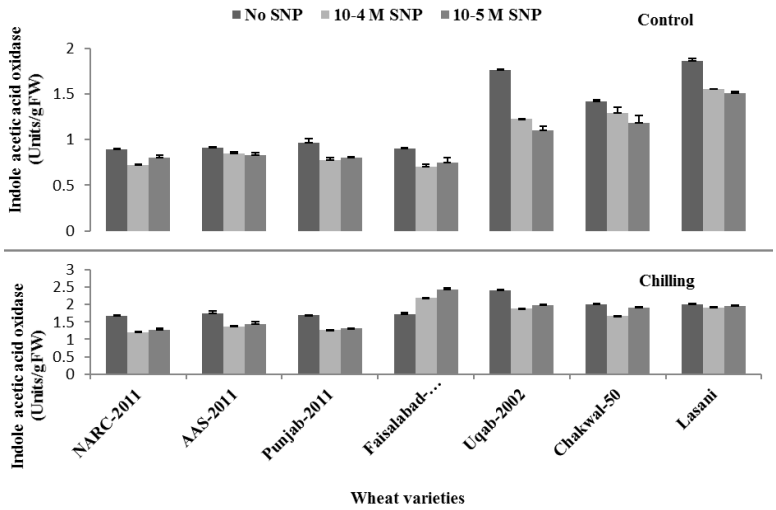

Fig. 7: Variations in indole acetic acid oxidase of wheat varieties as affected by SNP priming and chilling

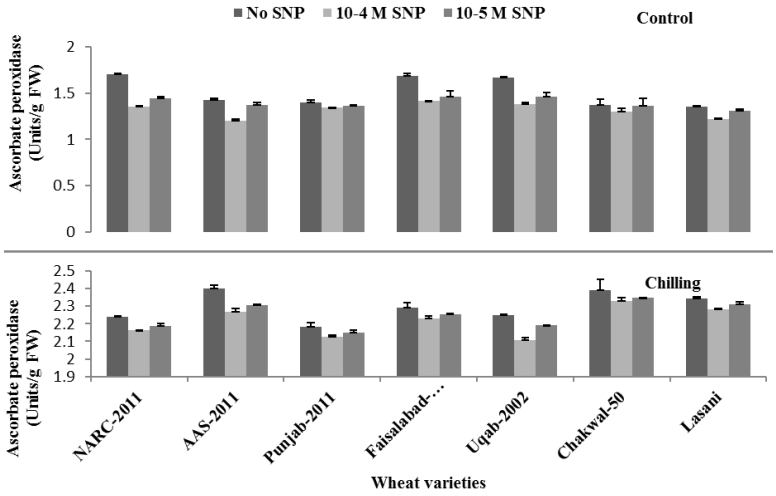

Fig. 8: Variations in ascorbate peroxidase of wheat varieties as affected by SNP priming and chilling

Ali *et al.* (2019) reported that priming exposure time also affected antioxidative defense mechanism variously in various varieties. If priming duration couldn't stimulate antioxidant enzyme activities in any plant species, then it stimulates other defense mechanisms like, proline, soluble sugars etc. for defense purpose. Nejadalimoradi *et al.* (2014) reported that SNP application could raise the activity of antioxidant enzymes in SNP treated plants compared to untreated one. In present study, many variations in antioxidant enzyme activities were observed from different wheat varieties under same treatment. This might be due to their varied tolerance to stress due to varied genetic makeup. Sairam *et al.* (2011) observed inconsistent antioxidant defense response in genotypes and could not notice any pattern *vis-à-vis* tolerant and susceptible genotypes. Shi *et al.* (2007) reported that NO enhanced the activity of CAT, APX and SOD in cucumber roots, and apoplastic H_2O_2 in NO-induced antioxidant defence. Increased SOD activity could enhance the ability of tissues to eliminate H_2O_2, explaining the lower level of H_2O_2 that was observed in NO-treated fruit. Treatment with nitric oxide significantly increased the activities of CAT, POD, SOD and APX under chilling stress (Ghorbani *et al.* 2018).

Conclusion

Genotypic differences in chilling stress tolerance were chiefly accredited to the capacity of wheat varieties to activate antioxidant defense. Capability of varieties to persuade the antioxidant response differs by different priming and growth conditions. Wheat varieties with better chilling tolerance than others sustained higher antioxidant enzyme activities that result in decreased oxidative damage and increased growth. However, SNP priming affected its antioxidant enzyme production *via* changing its metabolic reactions. Consequently, resistance against chilling induced

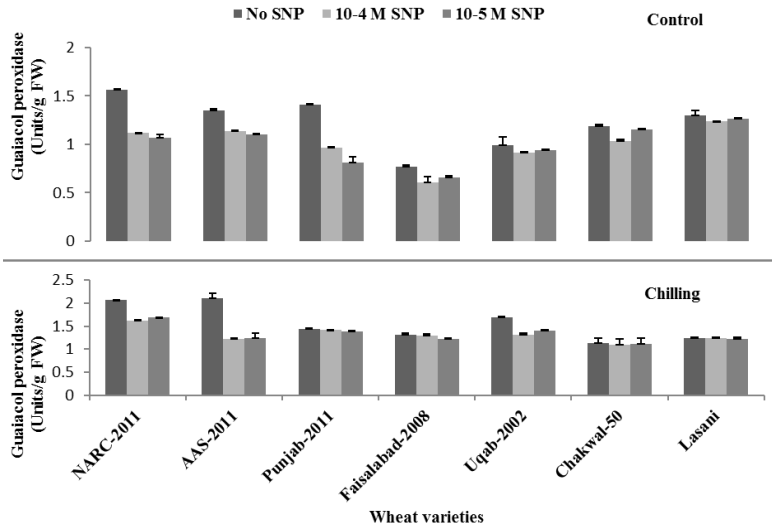

Fig. 9: Variations in guaiacol peroxidase of wheat varieties as affected by SNP priming and chilling

oxidative stress was primarily dependent on the genetic potential (superior antioxidant defense system) of wheat varieties. Germination speed, germination percentage, seedling growth and antioxidant status might be used as indices of chilling tolerance in wheat.

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